Amendments to the Specification

Please enter amendments (1) – (7) to the specification below.

(1) Please replace the existing Title of the Invention with the following *new* Title:

PROTECTION FROM ADVERSE EFFECTS ON THE PROSTATE

(2) Please insert the following *new* section heading immediately preceding the paragraph beginning at page 1, line 3, as follows:

FIELD OF THE INVENTION

(3) Please insert the following *new* section heading immediately preceding the paragraph beginning at page 1, line 7, as follows:

BACKGROUND OF THE INVENTION

(4) Please insert the following *new* section headings and paragraphs immediately preceding the paragraph beginning at page 3, line 2, as follows:

BRIEF DESCRIPTION OF THE DRAWNGS

Figure 1 is an analysis of *Shh* transcript distribution by in situ hybridisation. ^{33}P-labelled antisense and sense control riboprobes were hybridised to 7 μm paraffin-embedded sections of P0 male rat UGT. (A) Bright field image displaying the UR, VP and seminal vesicles (SV); (B) Dark field image of (A) showing *Shh* transcripts in the urethral epithelium (URE) and bud epithelium (VPE) of the developing VP. (C,D) show higher magnifications of (A,B) respectively. (D) shows *Shh* transcripts expressed in the VPE. (E) Bright field image showing the UR, VP, dorsal prostate (DP) and dorso-lateral prostate (DLP); (F) Dark field image of (E) revealing *Shh* transcripts in the URE and bud epithelium (DPE, DLPE and VPE) of the developing DP, DLP and VP lobes. (G,H) show higher magnifications of (E,F) respectively. Scale bar, 500 μm.

Figure 2 is analysis of *Shh* and *Ptc* transcripts in the male rat UGT, VP and UR by RNase protection assay. RNA was hybridised with ^{32}P-labelled antisense riboprobes for *Shh*, *Ptc*, and either *Cyclophilin* (*Cphn*) or *28S* as internal standards. Transcript levels were normalised to *Cphn* or *28S*. Figures below autoradiographs show % transcript abundance, relative to P0 UGT (A) or P0 VP (B,C), calculated as an average of three independent experiments. (A)

Shh and *Ptc* transcript levels in the developing male UGT. (B) *Shh* and *Ptc* transcript levels in the postnatal VP. (C) Comparison of *Shh* and *Ptc* transcript levels between postnatal VPs and URs.

Figure 3 is a comparison of *Shh* and *Ptc* transcript levels between male and female UGTs, and VPs and URs cultured -/+T. Figures below autoradiographs show % transcript abundance relative to P0 male UGT (A), or –T (B,C), calculated as an average of two experiments. (A) Comparison of *Shh* and *Ptc* transcript abundance between male and female UGTs. (B) Comparison of *Shh* and *Ptc* transcript levels between P0 VPs grown in vitro for six days with either no media supplement (-T), 10^{-8} M testosterone (+T), or for five days with 10^{-8} M testosterone followed by a one day treatment with 10^{-7} M cyproterone acetate (+T +CA). (C) Comparison of *Shh* and *Ptc* transcript levels between P0 female URs cultured for three days with either no media supplement (-T) or with 10^{-8} M testosterone (+T).

Figure 4 shows the effect of inhibition of SHH-signalling with anti-SHH antibody on VP growth. P0 VPs were grown in serum-free culture for five days in the presence or absence of 10^{-8} M T and/or 100 μg ml^{-1} anti-SHH antibody (5E1). (A) Wholemount images of cultured VPs. Scale bar, 1 mm. (B) Graph showing the mean two-dimensional areas of cultured VPs relative to –T +/- s.e.m.

Figure 5 shows the effect of inhibition of SHH-signalling with cyclopamine on VPs grown in vitro. VPs from P0 rats were grown in serum-free culture for six days in the presence or absence of 10^{-8} M T and/or 10^{-6} M Cy. (A) Whole mount images of VPs on day 6 of culture. Scale bar, 1 mm. (B) Graph showing the mean two-dimensional areas of cultured VPs relative to –T +/- s.e.m. (C) Graph showing the mean number of epithelial bud tips around the periphery of cultured VPs, expressed as a ratio to organ perimeter (mean buds per 1000 pixels perimeter +/- s.e.m.). (D) Graph showing the mean percentage of proliferating cells in epithelial buds +/- s.e.m. (Inset - BrdU incorporation was visualised by immunohistochemistry (green) and localised to the epithelium by immunohistochemistry for pan-cytokeratin (blue); nuclei were counterstained with propidium iodide (red).).

Figure 6 shows the effect of cyclopamine on the histology of prostatic epithelial ducts. Sections of VPs, cultured for six days in the presence or absence of T and/or Cy, were stained with Masson's trichrome. (A,B) organs grown –T, (C,D) organs grown –T +Cy; the addition

of Cy in the absence of T did not alter the appearance of epithelial tips. (E,F) organs grown +T; the addition of T resulted in canalisation of areas of ducts proximal to the UR (arrows). (G,H) organs grown +T +Cy; the addition of Cy in the presence of T resulted in canalisation of prostatic ducts throughout the organ, and the appearance of multiple lumens in ducts around the periphery (arrows). Scale bars, 50 μm.

Figure 7 shows the effect of Cy on prostatic epithelial cell differentiation. Paraffin sections of VPs, cultured for six days in the presence or absence of T and Cy, were stained with anti-p63 (A,C,E,G) or anti-cytokeratin 14 (B,D,F,H) antibodies to examine epithelial differentiation. (A,B) VPs grown with no media supplement (-T) exhibited p63 (A) and cytokeratin 14 (CK14) (B) staining throughout distal epithelial tips. p63 (C) and CK14 (D) expression was similar in VPs cultured with -T +Cy (C,D) to VPs cultured –T (A,B). (E,F) VPs cultured +T contained a mixture of distal epithelial tips with staining throughout, and distal tips where p63 (E) and CK14 (F) expression was confined to the basal cell layer. (G,H) In VPs grown +T +Cy, p63 (G) and CK14 (H) expression was confined to the basal cell layer in all tips. CK14 expression (H) was significantly reduced in +T +Cy VPs. Scale bar, 50 μm.

Figure 8 shows p63-immunostaining of human prostatic disease. Paraffin sections of human prostatic needle biopsies were stained with anti-p63 to visualise basal epithelial cells. (A) Normal (asterisk) or slightly hyperplastic ducts, showing only small discontinuities of the basal layer. (B) Expansion of the basal cell layer (arrow) in benign epithelial hyperplasia. (C) Benign cribiform hyperplasia with multiple lumens (asterisks), focal basal cell expansion and localised gaps. (D) High-grade PIN with a much more complex cribiform pattern, expansion of the size of the duct and regular gaps in the basal layer. Adjacent invasive carcinoma (arrow) is p63-negative. Scale bar, 100 μm.

Figure 9 shows prostatic induction in *Shh* null mice and rat UGS grown *in vitro* with cyclopamine. (A) Whole UGT of e17.5 *Shh* null male mouse displaying urogenital sinus (UGS), bladder (BL) and hindgut (HG). e17.5 *Shh* null male mouse UGS before (B) and after (C) growth *in vitro* for 5 days; arrows show prostatic buds. e16.5 male rat UGS grown *in vitro* for 7 days either without media supplement (D), +T (E) or +T+Cy (F); arrows show prostatic buds. Scale bars shown are 1 mm.

Figure 10 shows the effect of exogenous recombinant SHH protein on VPs grown *in vitro*. VPs from P0 rats were grown *in vitro* for 3 days -/+ T -/+ recombinant SHH. (A) Whole mount images of VPs on day 3 of culture. Scale bar, 1 mm. (B) Graph showing the mean 2D areas of cultured VPs relative to -T +/- s.e.m. (C) Graph showing the mean number of epithelial buds around the periphery of cultured VPs, expressed as a ratio to organ perimeter (mean buds per 1000 pixels perimeter +/- s.e.m.). (D) Graph showing the mean percentage of proliferating cells in distal epithelial buds (at 3 days) and surrounding mesenchyme (at 2 days) +/- s.e.m.

Figure 11 shows the structure of AY9944, triparanol, jervine, cyclopamine, fomatldine and cholesterol.

Figure 12 shows the structure of Compound A from WO 02/30462.

Figure 13 shows the structure of Compound B from WO 02/30462.

DETAILED DESCRIPTION OF THE INVENTION

(5) Please amend the paragraph beginning at page 8, line 8, as follows:

US Patent No ~~6,292,516 B1~~ 6,291,516 B1, incorporated herein by reference, also describes inhibitors of the SHH-signalling pathway. The compounds described in columns 27 to 35, including those of Formulae I, Ia, II, IIa, III, IIIa, IV, IVa, V, Va, VI, VIa, VII and VIIa are specifically incorporated herein by reference.

(6) Please insert the following *new* section heading immediately preceding the paragraph beginning at page 23, line 22, as follows:

EXAMPLES

(7) Please delete the paragraphs appearing at page 23, line 25, through page 27, line 20 (i.e., the description of drawings as originally presented).